（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1104-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader(500-520 nm)

Detection range：0.07-24 mmol/L

Elabscience® Tissue Free Cholesterol (FC) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 5

Materials prepared by users 5

Reagent preparation 5

Sample preparation 6

The key points of the assay 6

Operating steps 7

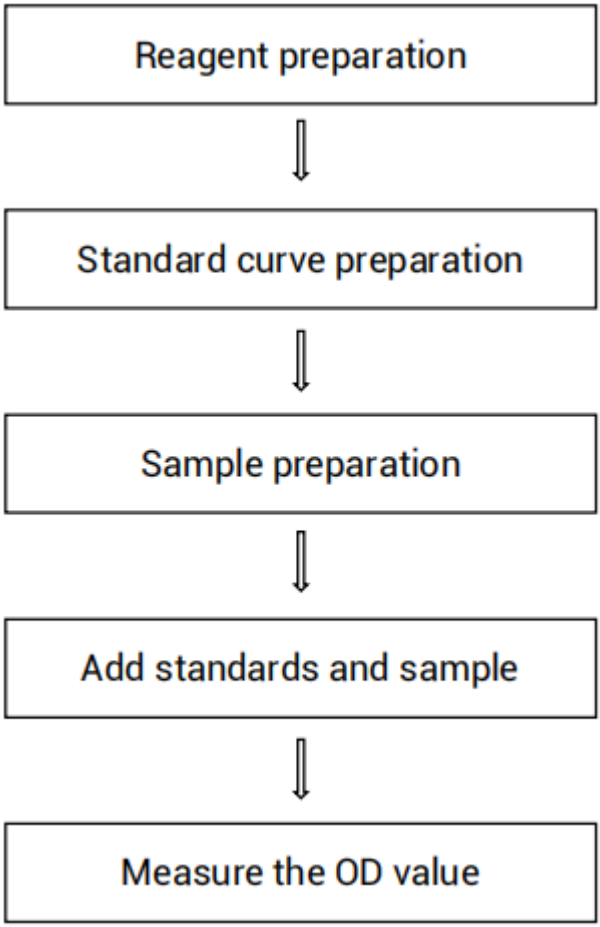
Calculation 7

Appendix I Performance Characteristics 8

Appendix П Example Analysis 10

Statement 11

Assay summary

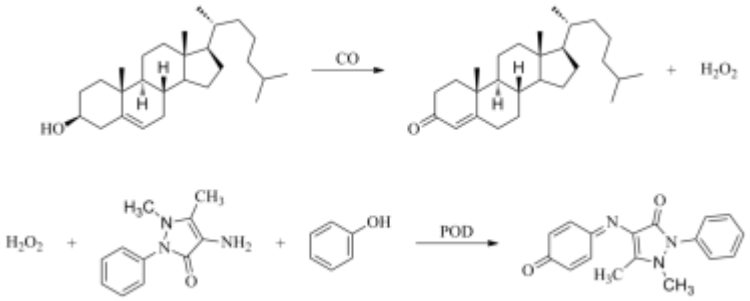


Intended use

This kit can be used to measure free cholesterol (FC) content in animal tissue samples.

Detection principle

Free cholesterol produces 4-cholestenone and hydrogen peroxide under the oxidation of cholesterol oxidase. In the presence of 4-aminoamylpyridine and phenol, peroxidase catalyze hydrogen peroxide to form red quinone compounds of benzoquinone imine phenizone. The color depth of the generated quinones is directly proportional to the cholesterol content.



Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	55 mL × 1 vial	55 mL × 2 vials	2-8°C, 12 months
Reagent 2	Enzyme Working Solution	15 mL × 1 vial	30 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	5.17 mmol/ L Cholesterol Standard	0.2 mL × 1 vial	0.2 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (500-520 nm, optimum wavelength: 510 nm)

Reagents:

Double distilled water

Reagent preparation

Equilibrate all the reagents to 25°C before use.

Sample preparation

① Sample preparation

Sample requirement: Reductive substances such as ascorbic acid and glutathione can't be added to the sample.

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000 × g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 8 h.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse heart tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Porcine liver tissue homogenate	1

Note: The diluent is extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Standard and samples should be added to the bottom of microplate.

② Enzyme working solution should be aliquoted and used to avoid contamination.

Operating steps

① Blank well: Add 5 μL of extraction solution into the wells.
Standard well: Add 5 μL of standard into the wells.
Sample well: Add 5 μL of sample into the wells.

② Add 250 μL of enzyme working solution into each well.

③ Incubate at 37°C for 10 min. Measure the OD value of each well at 510 nm.

Note: The reagent should be added to the bottom of microplate slowly to avoid bubble (bubbles will affect the test results).

Calculation

The sample:

Tissue samples:

$$\text{FC content (mmol/kg wet weight)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{m}{V}$$

[Note]

ΔA_1: $OD_{sample} - OD_{blank}$.

ΔA_2: $OD_{standard} - OD_{blank}$.

c: The concentration of standard , 5.17 mmol/L

f: Dilution factor of sample before test.

m: The weight of tissue sample, g.

V: The volume of of the extracting solution of tissue sample, mL

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% mouse heart tissue homogenate were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	5.17	10.34	15.51
%CV	1.9	1.65	1.5

Inter-assay Precision

Three 10% mouse heart tissue homogenate were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	5.17	10.34	15.51
%CV	5.2	4.2	3.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	5.17	10.34	15.51
Observed Conc. (mmol/L)	5.15	10.55	15.11
Recovery rate (%)	100	102	97

Sensitivity

The analytical sensitivity of the assay is 0.07 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

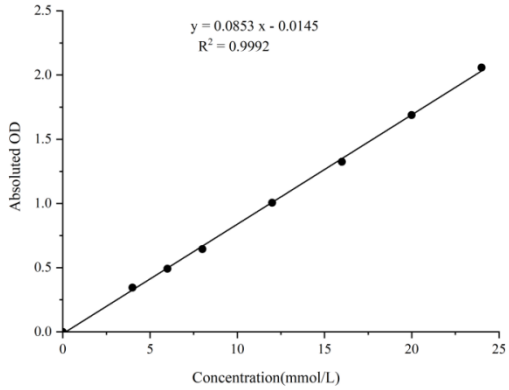
2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**4**	**6**	**8**	**12**	**16**	**20**	**24**
OD value	0.088	0.429	0.572	0.735	1.085	1.401	1.771	2.138
	0.089	0.437	0.588	0.732	1.103	1.425	1.782	2.155
Average OD value	0.088	0.433	0.58	0.734	1.094	1.413	1.776	2.146
Absolute OD value	0	0.344	0.492	0.645	1.006	1.324	1.688	2.058

Dilute 24 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 4, 6, 8, 12, 16, 20, 24 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**4**	**6**	**8**	**12**	**16**	**20**	**24**
24 mmol/L standard (μL)	0	100	150	200	300	400	500	600
Double distilled water (μL)	600	500	450	400	300	200	100	0



Appendix Π Example Analysis

Example analysis：

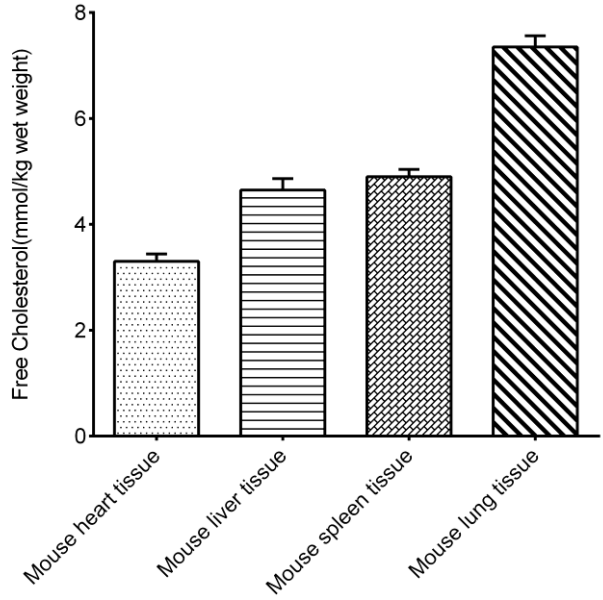
Take 5 μL of 10% mouse heart tissue homogenate into the well, and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample well is 0.114, the average OD value of the blank well is 0.089, the average OD value of the standard well is 0.419 and the calculation result is:

$$\text{FC content (mmol/kg wet weight)} = (0.114 - 0.089) \div (0.419 - 0.089) \times 5.17 \times 0.9 \div 0.1$$

$$= 3.52 \text{ mmol/kg wet weight}$$

Detect 10% mouse heart tissue homogenate, 10% mouse liver tissue homogenate, 10% mouse spleen tissue homogenate and 10% mouse lung tissue homogenate, according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.